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SHORT-CUT PROCEDURE FOR ERROR ESTIMATE IN LABORATORY STUDIES OF SYNERGISM IN INSECTICIDES

By F. M. Wadley

A circular has been published (3) giving a short graphic method for the study of synergism in insecticides. It involves the use of log probability plotting paper. Work based on the procedure described has led to various inquiries; and a supplement to this circular, carrying evaluations further, seems desirable.

For the further steps in evaluation proposed, it is advisable to transform percentages of mortality to probits, and concentrations to logarithms. These are plotted on ordinary cross-section paper. If concentrations of the mixture are reduced to the equivalents in terms of the principal insecticide and plotted, they can quickly be compared with results for the principal insecticide alone. Eye-fitted lines can be drawn, as in the circular cited. The horizontal distance between these lines is a measure of log difference of expected and actual curves (which is the logarithm of the ratio of concentrations needed for the same effect).

An estimate of standard error of this quantity is needed for satisfactory evaluation. Formulae for the calculation of the log ratio and its standard error are available (1). The formulae are very complex and require laborious calculations. However, it is found that the standard-error formula depends on several factors, which tend to vary but little in ordinary toxicological work. Thus a possibility exists of using a standardized estimate for quick preliminary evaluation.

Finney's formula (Eq. 8.13, p. 127) is formidable looking; it includes number of insects, weights, ratio of adjunct to principal toxicant, equivalence, regression coefficient, average kill, and sum of squares of deviations of log concentrations as its main factors. These factors are almost standardized in many laboratory tests. Concentrations are often spaced evenly on a log scale to give a series of mortalities centering around 50 percent. Number of insects used is determined by number of concentrations (often 3 or 4), and number of insects treated with each concentration. Weights vary with variation of mortalities around 50

percent. Error often does not exceed the theoretical value embodied in weights. Regression slopes are similar with toxicants of similar action. The curves should be parallel, or nearly so, for the evaluation to have fullest validity; and all the conditions named should hold approximately.

Basis for Error Estimates

Experiments lately discussed with Dr. J. J. Willaman (2) of the Department of Agriculture will illustrate the point. In nine of these tests there were usually four concentrations with about 30 insects per concentration for each material; mortalities centered about 50 percent, and regression coefficients ranged from about 3 to 6. Adjuncts as a rule had some toxicity, but not so much as the principal insecticide; ratios of ingredients were usually 50-50. Results from all these tests were subjected to the full analysis. The standard error of the log ratio varied somewhat, partly in response to small variations in conditions named, but averaged a little below 0.06. Trials showed that use of this value as standard was satisfactory in drawing preliminary conclusions. The average total number of insects per material was 118; the average regression coefficient about 4.5. Some material cited by Finney agrees with this error estimate fairly well, considering difference in numbers used.

Table 1 shows expected standard errors of log ratio under the general conditions described, with allowance for varying numbers and regression slopes. They are based on standard errors derived by the Finney formula from the actual cases mentioned.

Table 1.--Tentative standard errors for log ratio.

Regression coefficient	Total number of insects used per material								
	60	80	100	120	150	200	250	300	1000
2	0.20	0.17	0.15	0.14	0.13	0.11	0.10	0.09	0.05
3	.13	.11	.10	.09	.08	.07	.07	.06	.03
$4\frac{1}{2}$	.09	.08	.07	.06	.06	.05	.04	.04	.02
6	.07	.06	.06	.04	.04	.04	.03	.03	.02
8	.05	.04	.03	.03	.03	.03	.03	.02	.01
10	.04	.03	.03	.03	.03	.02	.02	.02	.01

Use of Standard Errors

It should be kept in mind that the standard errors in table 1 are approximate; also that even with exact determinations the fiducial limits may be uneven (1). Therefore a larger allowance than usual should be made. If an actual curve for a mixture is 3 standard errors or more from its expected value, to the side of lower concentration, there is strong evidence of synergism. Results showing much promise will probably be analyzed by the more detailed process, but the above approach will make possible quick preliminary evaluations. A graphic estimate cf regression is satisfactory in such tests.

For tests replicated on each of several days, as is desirable in more critical work, several courses may be followed. If the data are fairly homogeneous, they may be put together as if they were repeated determinations on the same day. The significance may be determined by consistency in separate evaluations from day to day. Some intermediate method of analysis, such as outlined in Finney's tables 27 and 28, may be brought in. In such replicated experiments it is desirable to have a complete set of tests on each day.

Numerical Example

Table 2.--Dosage-mortality data with principal insecticide,1/ adjunct, and a mixture.

Material	Number of insects used	Concentration, mg/cc			Log of concen- tration	Percent mortal- ity	Probit for mortality
		Insecti- cide	Adjunct	Insecticide equivalent			
Insecticide	23	4.7	-	-	0.67	35	4.61
	23	6.0	-	-	.78	78	5.77
	22	7.5	-	-	.88	82	5.92
Adjunct	22	-	6.7	-	.83	27	4.39
	21	-	10.4	-	1.02	83	5.95
	24	-	11.7	-	1.07	71	5.55
Insecticide plus Adjunct	24	2.3	.4	2.54	.40	29	4.45
	24	2.8	.5	3.10	.49	46	4.90
	24	3.4	.6	3.76	.57	67	5.44

1/ The principal insecticide here was nicotine, the adjunct was phthalonitrile, the insects were larvae of the diamondback moth.

From the graphic procedure outlined in ET 223 (3), it is found that the adjunct shows an insecticide equivalence of about 0.6. The insecticide equivalent of the mixtures is then calculated; for example, the calculation of the lowest concentration shows this equivalent as 2.30+(0.4 x 0.6), or 2.54. Logs of these equivalents are shown in the sixth column of the table.

The values for the insecticide and for the mixture are plotted in figure 1. The expected line for the mixture is that of the insecticide, since it has been put in terms of the insecticide. The actual line is that given by the logs and probits from the last three lines of the table. Eye-fitted lines are drawn. They do not fall so much in the same range as could be desired; but in the region of overlapping the mixture line is about 0.22 log units to the left of its expected value. The average regression coefficient estimated graphically is about 6, since the probit goes up about 0.6 with an increase of 0.1 in the log; the number of insects per material is about 70. By interpolation with these figures in table 1, a standard error of about 0.065 is inferred. The log ratio is about three times the standard error. The evidence for synergism is strong. The materials used have shown evidence of synergism in several cases.

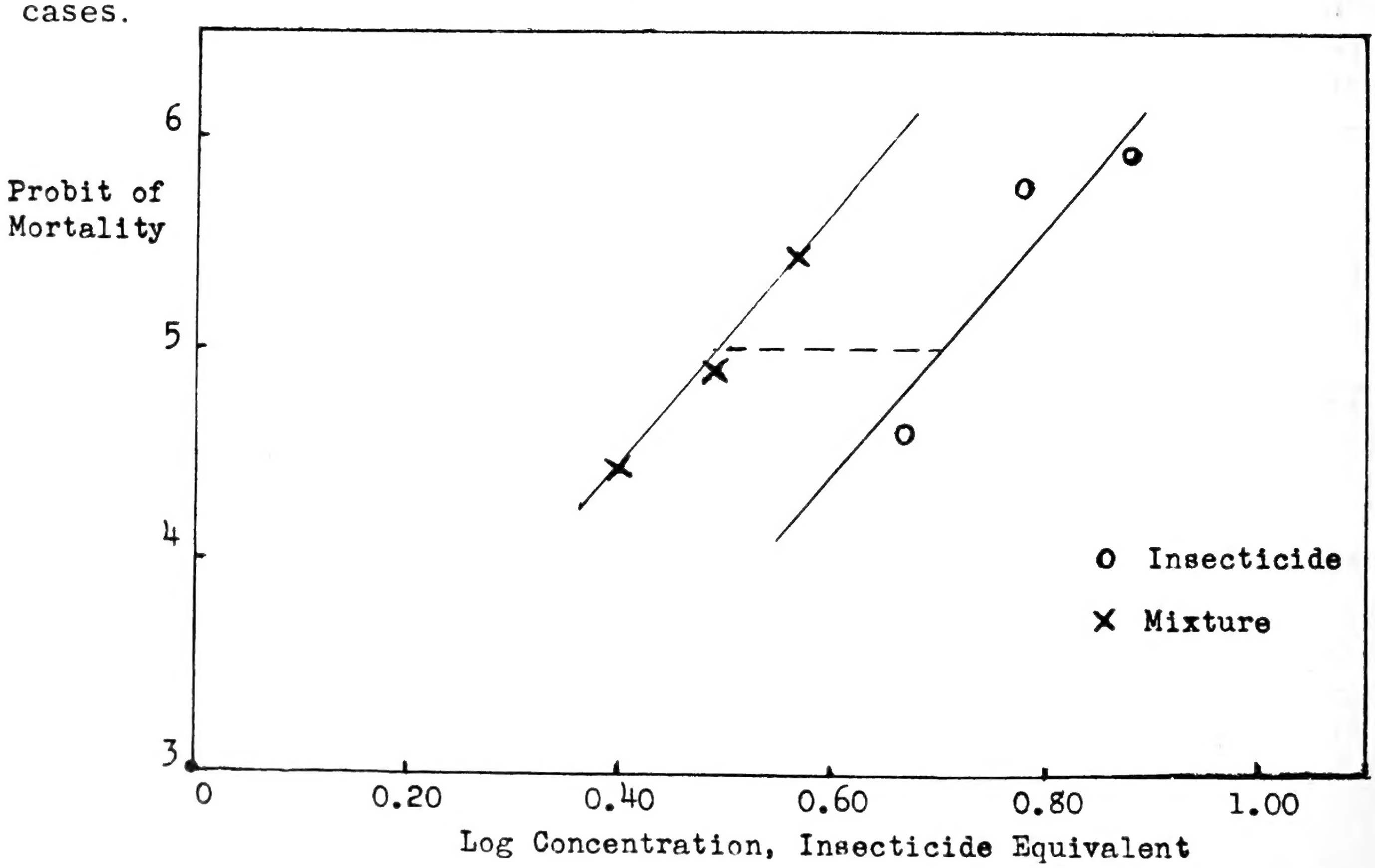

Figure 1. Graphic estimation of synergism, from data in table 2. (Insecticide line is expected line for mixture; mixture line gives actual result.)

Logarithms can be secured from many sources; any book of mathematical tables will include them. Probit tables are also widely available, and probits may, in fact, be read from any table of areas of the normal frequency curves. However, for the convenience of some workers, an abridged table of probits is appended for the range most used (table 3).

Table 3.--Probit values for percentages of mortality. (Add values at left and at top for percentage.)

	0	1	2	3	4	5	6	7	8	9
0	-	-	-	-	-	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Literature Cited

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